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(54) **RECOMBINANT *CORYNEBACTERIUM* HAVING 1,3-PDO PRODUCTIVITY AND REDUCED 3-HP PRODUCTIVITY, AND METHOD FOR PRODUCING 1,3-PDO BY USING SAME**

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C12N 15/67 (2006.01)

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CPC ***C12P 7/18*** (2013.01); ***C07K 14/34*** (2013.01); ***C12N 15/67*** (2013.01); ***C12N 15/77*** (2013.01); *C12Y 101/01006* (2013.01); *C12Y 101/01202* (2013.01); *C12Y 102/01003* (2013.01); *C12Y 207/0103* (2013.01)

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See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to recombinant *Corynebacterium* having 1,3-PDO productivity and reduced 3-HP productivity, and a method for producing 1,3-PDO by using same. When a *Corynebacterium glutamicum* variant according to the present invention is used, the productivity of 3-HP, which is a by-product, is inhibited by using low-cost glycerol as a carbon source, and thus 1,3-PDO can be produced with high efficiency.

3 Claims, 6 Drawing Sheets

Specification includes a Sequence Listing.

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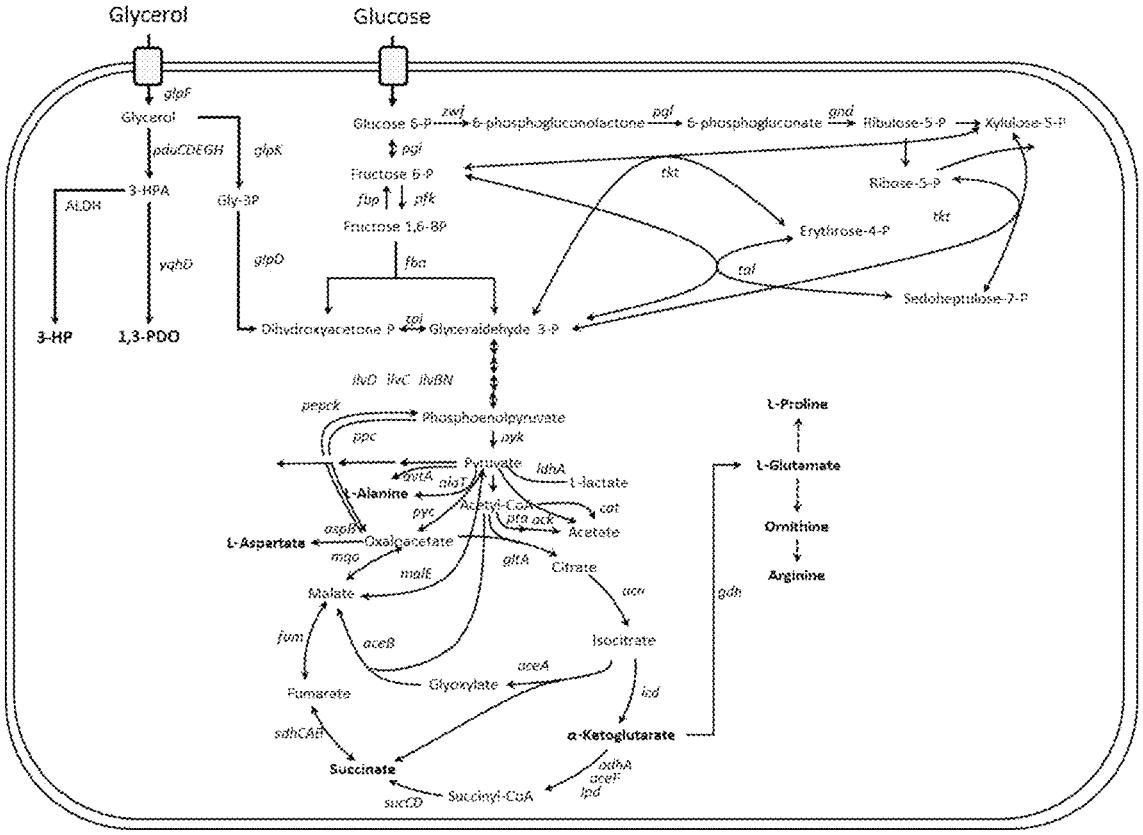
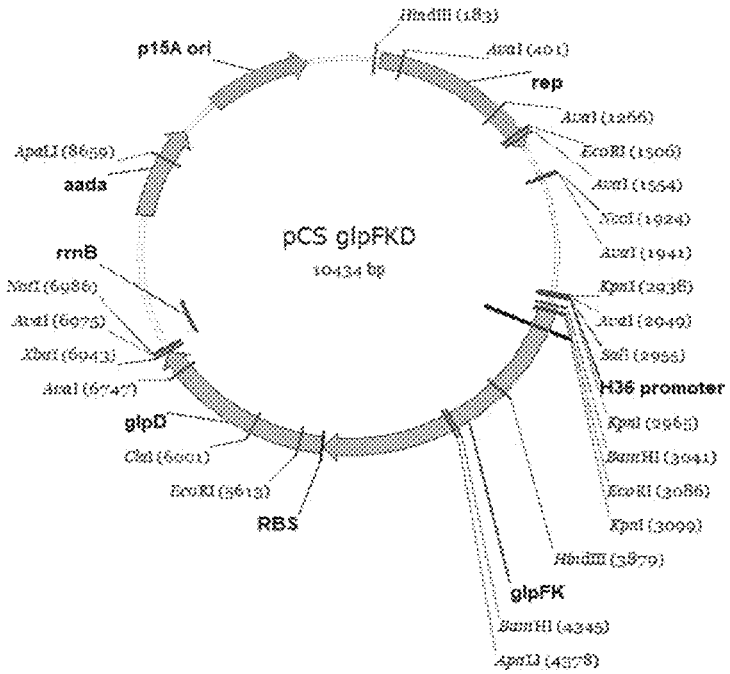
【Fig. 1】


Glycerol
Glucose
glpF
Glycerol
pduCDEGH
glpK
3-HPA
ALDH
Gly-3P
3-HP
1,3-PDO
yqhD
glpD
Glucose 6-P
zwf
6-phosphogluconolactone
pgl
6-phosphogluconate
gnd
Ribulose-5-P
Xylulose-5-P
pgi
Fructose 6-P
fbp
pfk
Fructose 1,6-BP
fba
tkt
Ribose-5-P
Erythrose-4-P
tal
Sedoheptulose-7-P
Dihydroxyacetone P
tpi
Glyceraldehyde 3-P
ilvD ilvC ilvBN
pepck
Phosphoenolpyruvate
ppc
pyk
Pyruvate
gpt A
alaT
L-Alanine
ldhA
L-lactate
Acetyl-CoA
pta ack
Acetate
cat
pyc
aspB
L-Aspartate
Oxaloacetate
mqo
malE
gltA
Citrate
Malate
acn
Isocitrate
fum
aceB
aceA
Glyoxylate
Fumarate
icd
sdhCAB
α-Ketoglutarate
Succinate
sucCD
Succinyl-CoA
odhA
aceF
lpd
L-Proline
L-Glutamate
Ornithine
Arginine
gdh

【Fig. 2】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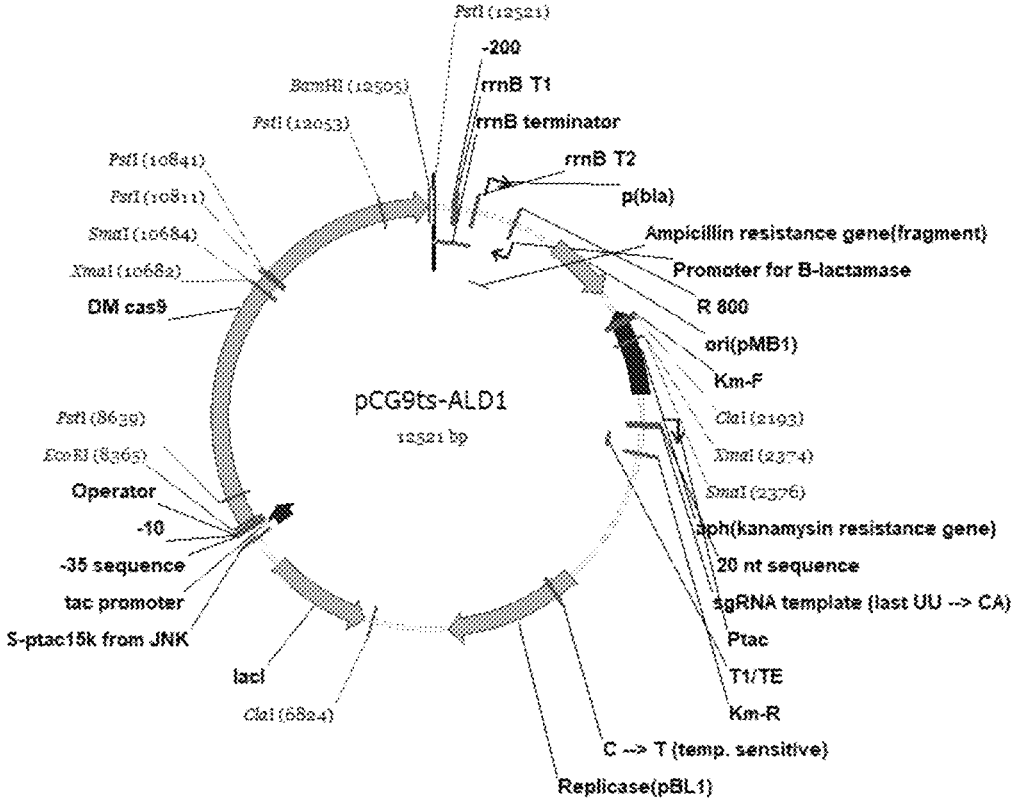
rep
aada
rrnB
glpD
RBS
glpFK
H36 promoter
pCS glpFKD
10434 bp

【Fig. 3】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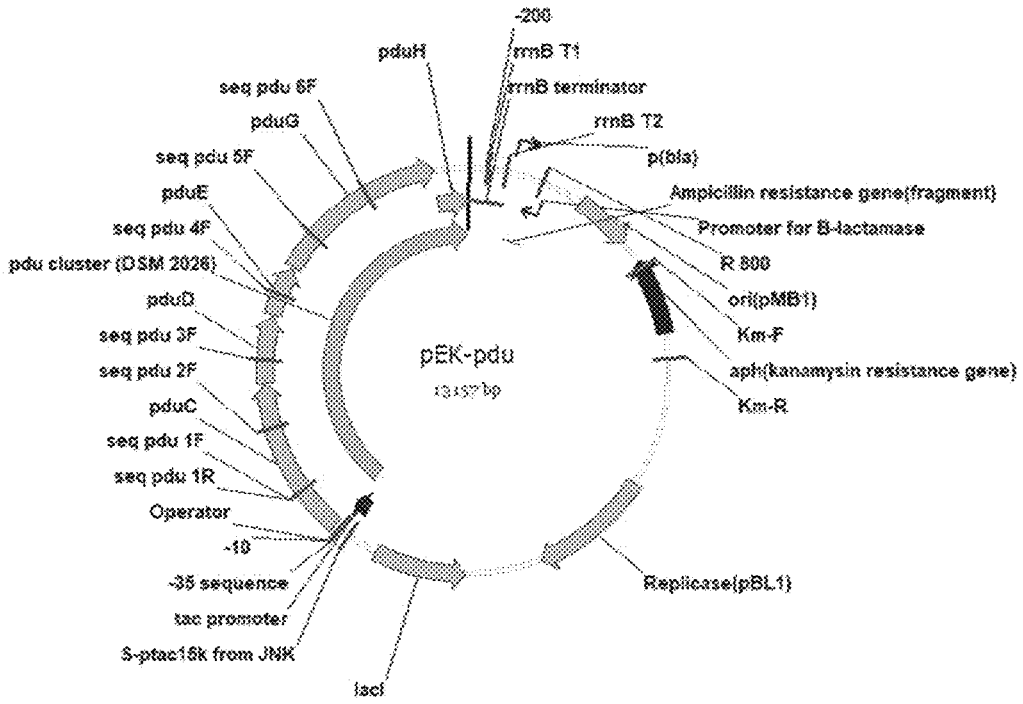
12321 bp
PstI (12321)
-200
rrnB T1
rrnB terminator
rrnB T2
p(bla)
Ampicillin resistance gene(fragment)
Promoter for B-lactamase
R 800
ori(pMB1)
Km-F
ClaI (2193)
XmaI (2374)
SmaI (2376)
aph(kanamysin resistance gene)
20 nt sequence
sgRNA template (last UU --> CA)
Ptac
T1/TE
Km-R
C --> T (temp. sensitive)
Replicase(pBL1)
ClaI (6824)
lacI
S-ptac15k from JNK
tac promoter
-35 sequence
-10
Operator
EcoRI (8365)
PstI (8639)
OM cas9
XmaI (10682)
SmaI (10684)
PstI (10811)
PstI (10841)
PstI (12053)
BamHI (12305)

【Fig. 4】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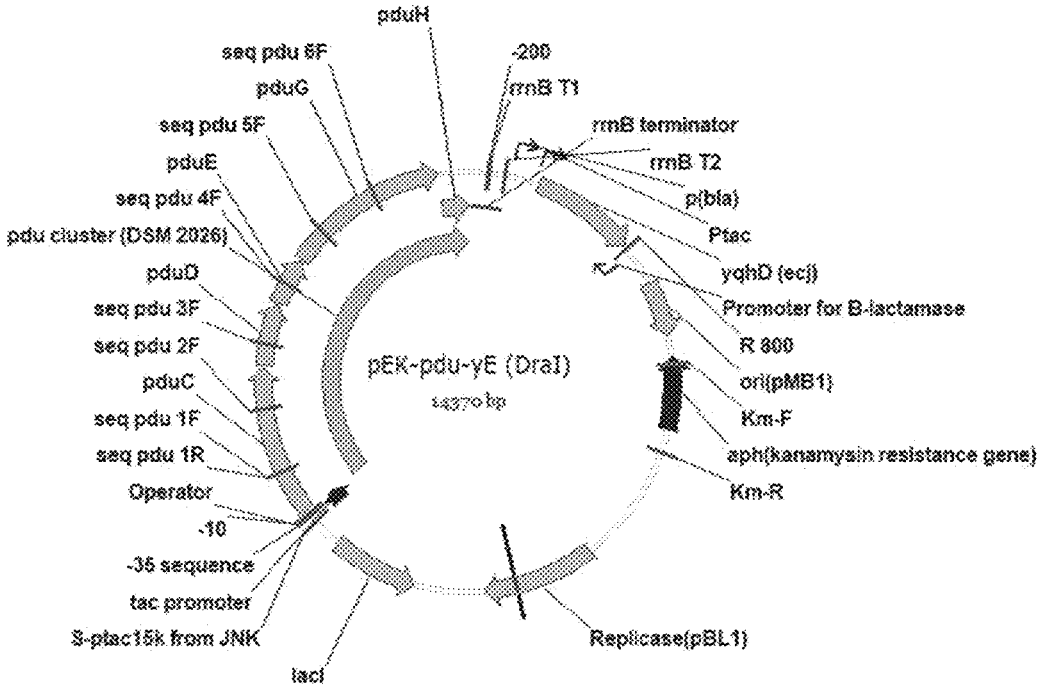
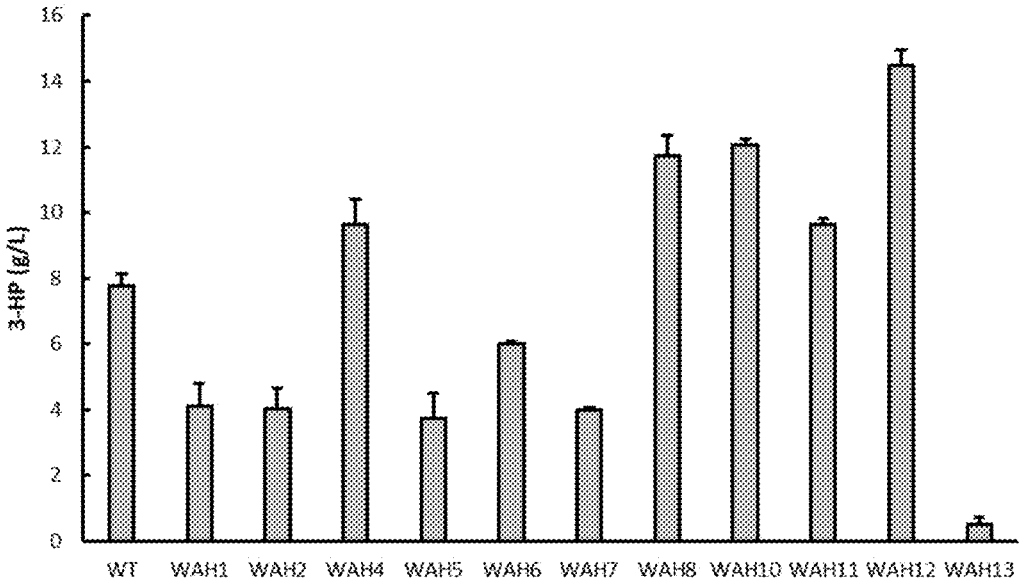
rrnB terminator
rrnB T2
p(bla)
Ampicillin resistance gene(fragment)
Promoter for B-lactamase
R 800
ori(pMB1)
Km-F
aph(kanamysin resistance gene)
Km-R
Replicase(pBL1)
lacI
S-ptac15k from JNK
tac promoter
-35 sequence
-10
Operator
seq pdu 1R
seq pdu 1F
pduC
seq pdu 2F
seq pdu 3F
pduD
pdu cluster (DSM 2026)
seq pdu 4F
pduE
seq pdu 5F
pduG
seq pdu 6F
pduH
pEK-pdu

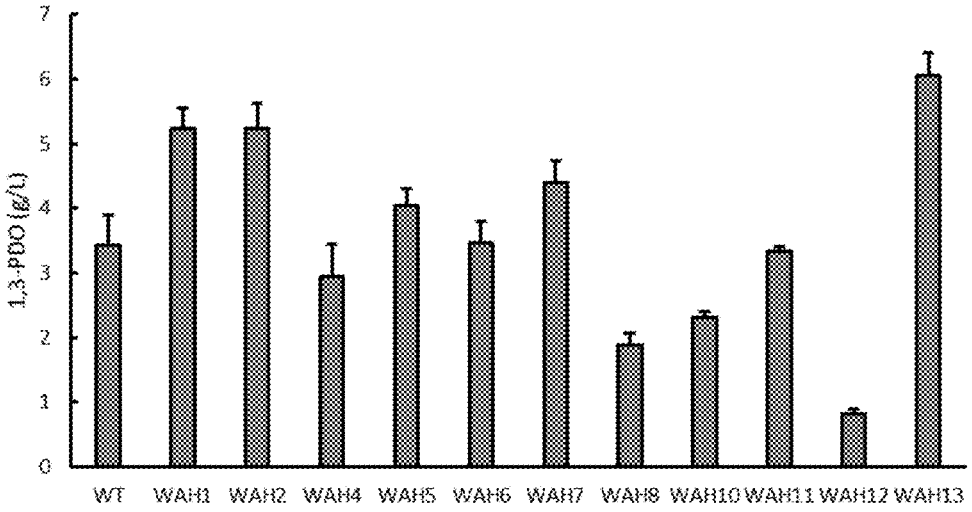
【Fig. 5】


pduH
seq pdu 6F
pduG
seq pdu 5F
pduE
seq pdu 4F
pdu cluster (DSM 2026)
pduD
seq pdu 3F
seq pdu 2F
pduC
seq pdu 1F
seq pdu 1R
Operator
-10
-35 sequence
tac promoter
S-ptac15k from JNK
lacI
-200
rrnB T1
rrnB terminator
rrnB T2
p(bla)
Ptac
yqhD (ecj)
Promoter for B-lactamase
R 800
ori(pMB1)
Km-F
aph(kanamysin resistance gene)
Km-R
Replicase(pBL1)
pEK-pdu-yE (DraI)

【Fig. 6】


3-HP (g/L)
16
14
12
10
8
6
4
2
0
WT
WAH1
WAH2
WAH4
WAH5
WAH6
WAH7
WAH8
WAH10
WAH11
WAH12
WAH13

【Fig. 7】


1,3-PDO (g/L)
7
6
5
4
3
2
1
0
WT
WAH1
WAH2
WAH4
WAH5
WAH6
WAH7
WAH8
WAH10
WAH11
WAH12
WAH13

RECOMBINANT *CORYNEBACTERIUM* HAVING 1,3-PDO PRODUCTIVITY AND REDUCED 3-HP PRODUCTIVITY, AND METHOD FOR PRODUCING 1,3-PDO BY USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a U.S. national phase under the provisions of 35 U.S.C. § 371 of International Patent Application No. PCT/KR19/04961 filed Apr. 24, 2019, which in turn claims priority under 35 U.S.C. § 119 of Korean Patent Application No. 10-2018-0058952 filed May 24, 2018. The disclosures of such international patent application and Korean priority patent application are hereby incorporated herein by reference in their respective entireties, for all purposes.

REFERENCE TO SEQUENCE LISTING SUBMITTED VIA EFS-WEB

This application includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "537_SeqListing_ST25.txt" created on Nov. 16, 2020 and is 54,316 bytes in size. The sequence listing contained in this .txt file is part of the specification and is hereby incorporated by reference herein in its entirety.

TECHNICAL FIELD

The present invention relates to a recombinant *Corynebacterium* having 1,3-PDO (1,3-propanediol) production ability and reduced (inhibited) 3-HP production ability, and a method for producing 1,3-PDO using the same, and more particularly, to a mutant microorganism having deleted or attenuated 3-HP production ability and producing 1,3-PDO from glycerol, wherein the mutant microorganism is produced by introducing a gene encoding a glycerol facilitator, a gene encoding glycerol kinase, a gene encoding glycerol dehydrogenase, a gene encoding glycerol dehydratase, a gene encoding glycerol reactivase and a gene encoding 1,3-PDO oxidoreductase into *Corynebacterium glutamicum*, and deleting or attenuating a gene encoding aldehyde dehydrogenase from the *Corynebacterium glutamicum*.

BACKGROUND ART

1,3-propanediol (1,3-PDO) is a chemical substance used as a monomer for the synthesis of polymers such as polyether, polyurethane and polytrimethylene terephthalate (PTT). Conventional methods mainly used for producing 1,3-PDO are chemical synthesis methods, and hydration of acrolein, hydroformylation of ethylene oxide in the presence of phosphine, or enzymatic conversion of glycerol may be used. These chemical production methods have limitations because they include high-cost and environmentally hazardous production processes (Lee et al., *Renewable and Sustainable Energy Reviews,* 42 (Supplement C): 963-972; U.S. Pat. No. 8,236,994 B2).

A biological method of producing 1,3-PDO using microorganisms is mainly performed using microorganisms such as *Klebsiella, Clostridia, Enterobacter, Citrobacter*, and Lactobacilli. In all of these methods, glycerol is directly converted to 1,3-PDO through two successive metabolic pathways of converting glycerol to 3-hydroxyproprionaldehyde (3-HPA) using glycerol dehydratase and then reducing the 3-HPA to 1,3-PDO using 1,3-PDO oxidoreductase (FIG. **1**). DuPont Inc. has already successfully commercialized 1,3-PDO by introducing the metabolic pathway into *E. coli*. However, there are disadvantages in which most microorganisms including *Escherichia coli* used for biosynthesis of 1,3-PDO are produced along with various byproducts, such as formate, acetate, lactate, ethanol, and 2,3-butanediol.

Corynebacterium glutamicum is a Gram-positive anaerobic bacterium which is widely used in fermentation processes for amino acid production. In addition, in order to produce various kinds of chemical substances and fuels using *Corynebacterium glutamicum*, a great deal of metabolic engineering research has been performed with the goal of realizing consumption of various types of carbon sources such as glucose and xylose, but there are few studies on the production of 1,3-PDO, and studies have reported simultaneous production of glutamic acid by promoting cell growth with glucose and producing 1,3-PDO with glycerol using glucose and glycerol as carbon sources in *Corynebacterium glutamicum* (Huang et al., *Scientific Reports,* 7: 42246, 2017).

However, 3-hydroxypropionaldehyde (3-HPA), which is an intermediate in the 1,3-PDO biosynthetic metabolic pathway, has a toxic effect when accumulated in cells, and acts as a precursor of 3-hydroxypropionic acid, which is one of the byproducts of 1,3-PDO. 3-HP is converted from 3-HPA through an aldehyde dehydrogenase enzyme, which has already been reported in the research to produce 3-HP by overexpressing GabD4(E209Q/E269Q), a mutant enzyme of GabD4, the gene encoding aldehyde dehydrogenase derived from *Cupriavidus necator*, in *Corynebacterium glutamicum* (Chen et al., *Metabolic Engineering,* 39:151, 2017). However, this is an effect caused by the overexpression of foreign enzymes, and there is no report associated with a gene that specifically accepts 3-HPA as a substrate, among aldehyde dehydrogenases that are still naturally present in *Corynebacterium glutamicum*, and that is involved in 3-HP biosynthesis.

Accordingly, as a result of extensive efforts to more efficiently produce 1,3-PDO through a biological pathway, the present inventors have found that 3-HP production ability was inhibited and thus 1,3-PDO was efficiently produced when culturing *Corynebacterium glutamicum* that was imparted with 1,3-PDO production ability by introducing a gene encoding glycerol dehydrogenase, a gene encoding glycerol dehydratase, a gene encoding glycerol reactivase and a gene encoding 1,3-PDO oxidoreductase, and at the same time, in which 3-HPA production ability is inhibited by deleting candidate aldehyde dehydrogenase genes present in *Corynebacterium glutamicum*, in order to produce a mutant *Corynebacterium glutamicum* having 1,3-PDO production ability and from which 3-HP production ability is inhibited or deleted. Based on this finding, the present invention was completed.

DISCLOSURE

Therefore, the present invention has been made in view of the above problems, and it is one object of the present invention to provide a mutant *Corynebacterium glutamicum* capable of efficiently producing 1,3-PDO due to inhibited 3-HP production ability.

It is another object of the present invention to provide a method of producing 1,3-PDO by culturing the mutant *Corynebacterium glutamicum*.

In accordance with one aspect of the present invention, the above and other objects can be accomplished by the provision of a mutant microorganism having deleted or attenuated 3-HP production ability and producing 1,3-PDO from glycerol, in which (i) a gene encoding a glycerol facilitator, (ii) a gene encoding glycerol kinase and a gene encoding glycerol dehydrogenase, (iii) a gene encoding glycerol dehydratase, (iv) a gene encoding glycerol reactivase and (v) a gene encoding 1,3-PDO oxidoreductase are introduced into *Corynebacterium glutamicum*, and a gene encoding aldehyde dehydrogenase is deleted or attenuated from the *Corynebacterium glutamicum*.

In accordance with another aspect of the present invention, there is provided a method of producing 1,3-PDO from glycerol, including (a) culturing the mutant microorganism in a glycerol-containing medium to produce 1,3-PDO, and (b) collecting the produced 1,3-PDO.

DESCRIPTION OF DRAWINGS

FIG. **1** is a schematic diagram illustrating the overall metabolic pathway of the mutant *Corynebacterium glutamicum* according to the present invention, including a 1,3-PDO biosynthetic metabolic pathway, a 3-HP biosynthetic metabolic pathway and a glycerol decomposition metabolic pathway.

FIG. **2** shows a pCSglpFKD recombinant vector into which glpF, glpK and glpD genes encoding the glycerol degradation metabolic pathway are inserted.

FIG. **3** shows a pCG-9ts-ALD1 recombinant vector produced to delete the NCgl0049 gene, among 13 aldehyde dehydrogenase candidates.

FIG. **4** shows a pEK-pdu recombinant vector produced by inserting the pduCDEGH gene cluster encoding glycerol dehydratase in order to construct a 3-HPA biosynthetic metabolic pathway.

FIG. **5** shows a pEK-pduyE recombinant vector produced by inserting the yqhD gene encoding *E. coli* 1,3-PDO oxidoreductase into the pEK-pdu vector in order to construct a 1,3-PDO biosynthetic metabolic pathway.

FIG. **6** shows the results of 3-HP production when using glycerol as a single carbon source by introducing pCSglpFKD and pEK-pduyE vectors into *Corynebacterium glutamicum* strains from which 11 types of aldehyde dehydrogenase are deleted.

FIG. **7** shows the results of 1,3-PDO production when using glycerol as a single carbon source by introducing pCSglpFKD and pEK-pduyE vectors into *Corynebacterium glutamicum* strains from which 11 aldehyde dehydrogenases are deleted.

BEST MODE

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as appreciated by those skilled in the field to which the present invention pertains. In general, the nomenclature used herein is well-known in the art and is ordinarily used.

In the present invention, a mutant *Corynebacterium glutamicum* having increased 1,3-PDO production ability was produced by inhibiting the production ability of 3-HP converted from the same precursor as 3-HPA, which is a precursor of 1,3-PDO, in the mutant *Corynebacterium glutamicum* in order to improve the 1,3-PDO yield using the mutant *Corynebacterium glutamicum* having 1,3-PDO production ability.

In the present invention, the mutant *Corynebacterium glutamicum* having 1,3-PDO production ability was produced by introducing a gene encoding glycerol dehydrogenase, a gene encoding glycerol dehydratase, a gene encoding glycerol reactivase and a gene encoding 1,3-PDO oxidoreductase into *Corynebacterium glutamicum*, which does not naturally have 1,3-PDO production ability.

The gene encoding glycerol dehydratase, the gene encoding glycerol reactivase and the gene encoding 1,3-PDO oxidoreductase used in the present invention are *Klebsiella pneumoniae*-derived pduCDEGH and *E. coli*-derived yqhD, and the 1,3-PDO production ability was determined after introducing the genes into *Corynebacterium glutamicum*.

Corynebacterium glutamicum used in the present invention is a microorganism that naturally allows for glycerol diffusion, but does not allow for cell growth when using a single carbon source. For this reason, *Corynebacterium glutamicum* enabling cell growth from a glycerol carbon source was produced by introducing a gene encoding a glycerol facilitator, a gene encoding glycerol kinase, and a gene encoding glycerol dehydrogenase.

In the present invention, glpF, glpK, and glpD derived from *E. coli* were respectively introduced as the gene encoding glycerol facilitator, the gene encoding glycerol kinase, and the gene encoding glycerol dehydrogenase.

In the present invention, *Corynebacterium glutamicum* introduced with the 1,3-PDO biosynthetic metabolic pathway produces 3-HP (3-hydroxypropionic acid) as a main byproduct in addition to 1,3-PDO, and 3-HP is converted from 3-HPA (3-hydroxypropionaldehyde), which is the same precursor as 3-PDO, by an aldehyde dehydrogenase enzyme. Enzymes that specifically react strongly to 3-HPA were identified from candidate enzymes of aldehyde dehydrogenases present in *Corynebacterium glutamicum*, and the effects obtained through in-vivo culture were determined.

Thus, in one aspect, the present invention is directed to a mutant microorganism having deleted or attenuated 3-HP production ability and producing 1,3-PDO from glycerol, wherein the mutant microorganism is produced by introducing (i) a gene encoding a glycerol facilitator, (ii) a gene encoding glycerol kinase and a gene encoding glycerol dehydrogenase, (iii) a gene encoding glycerol dehydratase, (iv) a gene encoding glycerol reactivase and (v) a gene encoding 1,3-PDO oxidoreductase into *Corynebacterium glutamicum*, and deleting or attenuating a gene encoding aldehyde dehydrogenase from the *Corynebacterium glutamicum*.

In the present invention, the gene encoding aldehyde dehydrogenase, which is an enzyme involved in providing the mutant *Corynebacterium glutamicum* having inhibited 3-HP production ability, includes 11 candidate genes present in *Corynebacterium glutamicum*, namely NCgl0049, NCgl0157, Ncgl0437, NCgl0463, NCgl0521, NCgl0523, NCgl0900, NCgl2272, NCgl2578, NCgl2619, and NCgl2698.

In the present invention, a change in 3-HP production due to deletion of the 11 candidate genes selected for 3-HP biosynthesis inhibition was determined, and a mutant *Corynebacterium glutamicum* having increased 1,3-PDO production was produced.

In the present invention, at least one of the genes encoding aldehyde dehydrogenase may be deleted or attenuated.

In the present invention, the gene encoding the glycerol facilitator, the gene encoding glycerol kinase, and the gene encoding glycerol dehydrogenase may be glpF, glpK and glpD, respectively, and the gene encoding glycerol dehy-

dratase, the gene encoding glycerol reactivase, and the gene encoding 1,3-PDO oxidoreductase may be pduCDEG or yqhD.

In the present invention, the introduced genes may be overexpressed by a strong promoter selected from the group consisting of tac, trc and tuf.

As used herein, the term "intrinsic activity" refers to the activity of an enzyme that a microorganism innately has in an unmodified state, the expression "modified to have enhanced activity compared to intrinsic activity" means that an activity is newly introduced or improved compared to the enzymatic activity before modification.

As used herein, the term "enhancement in enzymatic activity" includes not only having effects beyond original functions through new introduction of activity of enzymes or improvement thereof, but also increased enzymatic activity based on an increase in endogenous gene activity, amplification of endogenous genes due to internal or external factors, deletion of inhibitory regulatory factors of the gene expression, an increase in the number of copies of genes, introduction of genes from external sources, modification of expression regulation sequences, in particular, promoter replacement or modification, and increased enzymatic activity due to gene mutations.

As used herein, the term "modified to have enhanced activity compared to intrinsic activity" means a state in which the activity of the microorganism after manipulation is increased compared to the activity of the microorganism before manipulation, such as the introduction of genes exhibiting activity or an increased number of copies of the corresponding gene, and deletion of inhibitory regulatory factors of gene expression or modification of expression regulation sequences, for example, the use of enhanced promoters.

As used herein, the term "deletion" encompasses cases in which a gene is not expressed through a method of mutation, replacement or deletion of a part or the entirety of the base of the gene and cases in which the enzymatic activity thereof is not expressed even though the gene is expressed, and includes all operations for blocking biosynthetic pathways that the enzyme of the corresponding gene mediates.

As used herein, the term "overexpression" refers to expression at a level higher than the level at which the corresponding gene in the cell is expressed in a normal state, and includes increases in expression levels by replacing promoters of genes present on the genome with stronger promoters or cloning the corresponding gene into the expression vector to transform cells therewith.

As used herein, the term "vector" means a DNA product containing a base sequence of a polynucleotide encoding a target protein operably linked to a suitable control sequence so as to express the target protein in a suitable host. The control sequence includes a promoter capable of initiating transcription, any operator sequence for controlling such transcription, a sequence encoding a suitable mRNA ribosomal binding site, and a sequence for controlling termination of transcription and translation. After the vector is transformed into a suitable host cell, it may be replicated or perform functions independent of the host genome, and may be integrated with the genome.

Since the plasmid is the most commonly used type of vector, the terms "plasmid" and "vector" may be used interchangeably throughout the specification of the present invention. For the purpose of the present invention, a plasmid vector is preferably used. A typical plasmid vector that can be used for this purpose includes (a) a replication origin to efficiently conduct replication such that several hundred plasmid vectors are included in each host cell, (b) an antibiotic resistance gene to screen a host cell transformed with the plasmid vector, and (c) a restriction enzyme cleavage site into which a foreign DNA fragment is inserted. Even if an appropriate restriction enzyme cleavage site is not present, the vector and foreign DNA can be easily ligated using a synthetic oligonucleotide adapter or a linker according to a conventional method.

After ligation, the vector should be transformed into an appropriate host cell. The host cells preferred in the present invention are prokaryotic cells. Suitable prokaryotic host cells include *E. coli* DH5a, *E. coli* JM101, *E. coli* K12, *E. coli* W3110, *E. coli* X1776, *E. coli* XL-1 Blue (Stratagene), *E. coli* B, *E. coli* B21 and the like. However, *E. coli* strains such as FMB101, NM522, NM538 and NM539, as well as other prokaryotic species and genera, and the like, can also be used. In addition to the *E. coli* mentioned above, strains of the genus *Agrobacterium*, such as *Agrobacterium* A4, *Bacillus* strains such as *Bacillus subtilis*, other enterobacteria such as *Salmonella typhimurium* or *Serratia marcescens*, and various strains of the genus *Pseudomonas* can be used as host cells.

Transformation of prokaryotic cells can be easily carried out using a calcium chloride method described in Section 1.82 of Sambrook et al., supra. Alternatively, electroporation (Neumann, et al., EMBO J., 1: 841, 1982) can be used for transformation of these cells.

The vector used for overexpression of the gene according to the present invention may be any expression vector known in the art, and is preferably a pET-based vector (Novagen). When cloning is performed using the pET-based vector, histidine groups are bonded to the ends of the expressed protein, so that the protein can be effectively purified. The expressed protein can be isolated from the cloned gene through a general method known in the art, and can be specifically isolated using a chromatographic method using Ni-NTA His-conjugated resin (Novagen). In the present invention, the recombinant vector may be pET-SLTI66, and the host cell may be *E. coli* or *Agrobacterium*.

As used herein, the term "expression control sequence" means a DNA sequence essential for the expression of a coding sequence operably linked to a particular host organism. Such a control sequence includes promoters for conducting transcription, any operator sequences for controlling such transcription, sequences for encoding suitable mRNA ribosome-binding sites, and sequences for controlling the termination of transcription and translation. For example, control sequences suitable for prokaryotes include promoters, optionally operator sequences and ribosome-binding sites. Control sequences suitable for eukaryotic cells include promoters, polyadenylation signals, and enhancers. The factor that has the greatest impact on the expression level of a gene in a plasmid is the promoter. SRα promoters, cytomegalovirus-derived promoters and the like are preferably used as promoters for high expression. Any of a wide variety of expression control sequences may be used for the vector in order to express the DNA sequences of the present invention. Useful expression control sequences include, for example, early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, T3 and T7 promoters, the major operator and promoter regions of phage lambda, control regions of fd code proteins, promoters of 3-phosphoglycerate kinase or other glycol lyases, promoters of the phosphatase, such as Pho5, promoters of yeast alpha-mating systems and other sequences known to control gene expression of prokaryotic or eukary-

otic cells or viruses and various combinations thereof. The T7 promoter may be useful for expressing proteins of the present invention in *E. coli*.

When a nucleic acid sequence is aligned with another nucleic acid sequence based on a functional relationship, it is "operably linked" thereto. This may be gene(s) and control sequence(s) linked in such a way so as to enable gene expression when a suitable molecule (e.g., a transcriptional activator protein) is linked to the control sequence(s). For example, DNA for a pre-sequence or secretory leader is operably linked to DNA for a polypeptide, when expressed as a pre-protein involved in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence when it affects the transcription of the sequence; or a ribosome-binding site is operably linked to a coding sequence when it affects the transcription of the sequence; or the ribosome-binding site is operably linked to a coding sequence when positioned to facilitate translation. Generally, "operably linked" means that the linked DNA sequence is in contact therewith, and a secretory leader is in contact therewith and is present in the reading frame. However, the enhancer need not be in contact therewith. The linkage of these sequences is carried out by ligation (linkage) at convenient restriction enzyme sites. When no such site exists, a synthetic oligonucleotide adapter or a linker according to a conventional method is used.

As used herein, the term "expression vector" commonly refers to a recombinant carrier, into which a fragment of heterologous DNA is inserted, and generally means a fragment of double-stranded DNA. Herein, the heterologous DNA is xenogenous DNA that is not naturally found in the host cell. Once an expression vector is present in a host cell, it can replicate independently of the host chromosomal DNA, and several copies of the vector and inserted (heterologous) DNA thereof can be produced.

As is well known in the art, in order to increase the expression level of a transgene in a host cell, the gene should be operably linked to transcriptional and translational expression control sequences that function in a selected expression host. Preferably, the expression control sequence and the corresponding gene are included in one expression vector containing both a bacterial selection marker and a replication origin. When the expression host is a eukaryotic cell, the expression vector should further include a useful expression marker in the eukaryotic expression host.

The host cell transfected or transformed with the expression vector described above constitutes another aspect of the present invention. As used herein, the term "transfection" means introducing DNA into a host and making the DNA replicable using an extrachromosomal factor or chromosomal integration. As used herein, the term "transformation" means that an expression vector is accommodated in the host cell, regardless of whether or not any coding sequence is actually expressed.

It should be understood that not all vectors and expression control sequences function identically in expressing the DNA sequences of the present invention. Likewise, not all hosts function identically for the same expression system. However, those skilled in the art will be able to make appropriate selection from among a variety of vectors, expression control sequences and hosts without excessive burden of experimentation and without departing from the scope of the present invention. For example, selection of a vector should be carried out in consideration of a host because the vector should be replicated therein. The number of replications of the vector, the ability to control the number of replications, and the expression of other proteins encoded by the corresponding vector, such as the expression of antibiotic markers, should also be considered. In selecting the expression control sequence, a number of factors should be considered. For example, the relative strength of the sequence, controllability, and compatibility with the DNA sequences of the present invention should be considered, particularly in relation to possible secondary structures. The single-cell host may be selected in consideration of factors such as the selected vector, the toxicity of the product encoded by the DNA sequence of the present invention, secretion characteristics, the ability to accurately fold proteins, culture and fermentation factors, and ease of purification of the product encoded by the DNA sequence according to the present invention from the host. Within the scope of these factors, those skilled in the art can select various vector/expression control sequences/host combinations capable of expressing the DNA sequences of the present invention in fermentation or large animal cultures. As a screening method for cloning cDNA of proteins through expression cloning, a binding method, a panning method, a film emulsion method or the like can be applied.

Hereinafter, the present invention will be described in more detail with reference to examples. However, it will be obvious to those skilled in the art that these examples are provided only for illustration of the present invention and should not be construed as limiting the scope of the present invention.

In the following Examples, only genes derived from a specific strain are given as examples of genes to be introduced, but it will be apparent to those skilled in the art that any genes may be used without limitation, as long as they are expressed in host cells into which they are to be introduced and exhibit the same activity.

Example 1: Production of pCSglpFKD Vector for Production of Mutant *Corynebacterium glutamicum* Capable of Growing Using Glycerol as Single Carbon Source

1-1: Production of pCSglpFKD Vector for Constructing Glycerol Decomposition Metabolic Pathway

Corynebacterium glutamicum is known to be unable to grow cells using glycerol as a single carbon source. Therefore, in order to construct the glycerol decomposition metabolic pathway, the gene encoding the enzyme derived from *E. coli* W3110 and responsible for the glycerol decomposition metabolic pathway was first expressed using the *Corynebacterium glutamicum* shuttle vector, pCES208s-H36-S3.

PCR was performed using the chromosomal DNA of *E. coli* W3110 (ATCC 39936) as a template and primers of SEQ ID NOS: 1 and 2 to obtain glpFK gene fragments encoding glycerol facilitator and glycerol kinase operon enzymes, and PCR was performed using primers of SEQ ID NOS: 3 and to obtain glpD gene fragments encoding glycerol-3-phosphate dehydrogenase. In order to ligate the glpFK gene fragment with the glpD gene fragment, overlapping PCR was performed using the primers of SEQ ID NOS: 1 and 4 to produce a glpFKD gene fragment (SEQ ID NO: 53). In order to linearize the pCES208s-H36-S3 vector ((the vector (SEQ ID NO: 21) obtained by replacing the Km antibiotic of pCES208-H36 vector (Korean Patent Laid-open Publication No. 10-2013-0022691, or Yim S. S. et al., *Biotechnol. Bioeng.*, 110:2959, 2013, SEQ ID NO: 54)) with the antibiotic spectinomycin, PCR was performed using primers of SEQ ID NOS: 5 and 6, and a pCSglpFKD vector

was constructed using the produced glpFKD gene fragment and a Gibson assembly method (FIG. **2**).

TABLE 1

Primers for producing pCSglpFKD vector

SEQ ID NO	Nucleotide sequence
SEQ ID NO: 1	5'-TTGGTTGGTAGGAG TAGCATGGGATCCATGA GTCAAACATCAACCT T-3'
SEQ ID NO: 2	5'-GTTTCCATCTATAT CTCCTTTTATTCGTCGT GTTCTTCCC-3'
SEQ ID NO: 3	5'-AAGGAGATATAGAT GGAAACCAAAGATCTGA T-3"
SEQ ID NO: 4	5'-TAATTATAATGGCCGGC TGGGCCTCTAGAGTTAC GACGCCAGCGATAAC C-3"
SEQ ID NO: 5	5'-TCTAGAGGCCCAGC CGGCCATTATAATTA G-3'
SEQ ID NO: 6	5'-GGATCCCATGCTAC TCCTACCAACCAAGG T-3'

Example 2: Production of Aldehyde Dehydrogenase Deletion Vector for Inhibiting 3-HP Biosynthesis

When 1,3-PDO is produced from glycerol, 3-HPA, a produced precursor, is converted to 3-HP through the aldehyde dehydrogenase enzyme present in the cell. However, no enzyme that catalyzes the reaction to accept the precursor as a substrate in *Corynebacterium glutamicum* has been reported. Therefore, in order to identify the aldehyde dehydrogenase enzyme that mediates the reaction and delete the gene encoding the enzyme from the genome of the strain to thereby inhibit 3-HP biosynthesis, first, 13 kinds of aldehyde dehydrogenase enzymes present in *Corynebacterium glutamicum* were selected (Table 2).

Then, in order to confirm the inhibitory effect of 3-HP biosynthesis by deletion of the genes (SEQ ID NOS: 56 to 68) encoding the 13 kinds of aldehyde dehydrogenase enzymes, first, the strain including the pTacCC1-HrT vector transformed into *Corynebacterium glutamicum* was produced (Cho et al., *Metabolic Engineering,* 42: 157-167, 2017). Then, i) pCG9ts series each containing sgRNA sequences of 12 types of genes, and ii) ssODNs each binding to 13 kinds of genes for the produced *Corynebacterium glutamicum* strain were produced to perform gene deletion in *Corynebacterium glutamicum.*

2-1: Production of pCG9ts-Series Vectors Containing sgRNA Guide Sequences of 13 Kinds of Genes

First, using the online program CRISPy-web (Blin et al., *Synthetic and Systems Biotechnology,* 1(2):118-121, 2016), which analyzes the non-specific target of the guide sequence of sgRNA and provides the optimal sgRNA guide sequence, the following optimal guide sequences having a low off-target effect were selected (Table 2).

TABLE 2

sgRNA guide sequences for 13 arbitrary kinds of aldehyde dehydrogenase using CRISPy-web

Guide sequence	Target gene	sgRNA guide sequence
SEQ ID NO: 7	NCg10049	TTCGTGGACTAAGAAACGGT
SEQ ID NO: 8	NCg10157	TGCAGGATTGTAGACAGGAG
SEQ ID NO: 9	NCg10248	TTCACCTCAGAGACGATTAG
SEQ ID NO: 10	NCg10437	TGTTTGCTAAAGAGTAGGAA
SEQ ID NO: 11	NCg10463	AACTCCCCGCGAAAGATCCG
SEQ ID NO: 12	NCg10521	TTCGGAGACACACACATGTA
SEQ ID NO: 13	NCg10523	CCAGTGACTTTAGAGCTAGG
SEQ ID NO: 14	NCg10900	CCAACTGATATCGTGCTGTA
SEQ ID NO: 15	NCg11526	GTCGCCAGTGTATGCGTGAA
SEQ ID NO: 16	NCg12272	GCGCAGCAAAGCTACGTTTC
SEQ ID NO: 17	NCg12578	ATCGTCGTAAGGATTGATAT
SEQ ID NO: 18	NCg12619	GAGGTTATAGCGCCATTTAC
SEQ ID NO: 19	NCg12698	CTTGCCAATCCGATTAGAGC

In order to produce pCG9ts-series vectors including the sgRNA guide sequences (SEQ ID NOS: 7 to 19), DNA fragments targeting the NCgl0049 gene and encoding the sgRNA-T1/TE sequence (Korea Patent Application No. 2017-0042124; Cho et al., *Metabolic Engineering,* 42: 157-167, 2017) were amplified using pUC19-sgRNA vector (Korean Patent Application No. 2017-0042124; Cho et al., *Metabolic Engineering,* 42: 157-167, 2017, SEQ ID NO: 55) as a template and primers of SEQ ID NOS: 20 and 23. The amplified DNA fragments were amplified again through PCR using the primers of SEQ ID NOS: 21 and 22. After a pEKts-Cas9 vector (Korean Patent Application No. 2017-0042124; Cho et al., *Metabolic Engineering,* 42: 157-167, 2017, SEQ ID NO: 66) was treated with a StuI enzyme, a pCG9ts-ALD1 vector expressing the sgRNA targeting the NCgl0049 gene together with the Cas9 protein was finally produced through Gibson assembly with the amplified fragment. Then, fragments targeting genes encoding each of 13 arbitrary kinds of enzymes were produced in the same manner as above (the same in the case of SEQ ID NOS: 20, 21 and 22; PCR was conducted in the order of SEQ ID NOS: 24 to 35 for respective genes) to produce pCG9ts-ALD2, pCG9ts-ALD3, pCG9ts-ALD4, pCG9ts-ALD5, pCG9ts-ALD6, pCG9ts-ALD7, pCG9ts-ALD8, pCG9ts-ALD9, pCG9ts-ALD10, pCG9ts-ALD11, pCG9ts-ALD12 and pCG9ts-ALD13 vectors.

TABLE 3

Primers for amplifying sgRNA-T1/TE fragments

SEQ ID NO	Nucleotide sequence
SEQ ID NO: 20	TATA**GATATC**CCGCGGTATATTAA TTAATATAAACGCAGAAAGGCCC
SEQ ID NO: 21	TGGATGATGGGGCGATTCAGGtatag atatcTTGACAATTAATCATCGGCT

TABLE 3-continued

Primers for amplifying sgRNA-T1/TE fragments	
SEQ ID NO	Nucleotide sequence
SEQ ID NO: 22	AAGGTGTTGCTGACTCATACCAGGTA TAGATATCCCGCGGTATA

TABLE 4

Primers for producing pCG9ts-series vectors and 13 randomly selected enzymes

SEQ ID NO	Gene	Annotation	Nucleotide sequence
SEQ ID NO: 23	NCg10049	SSADH	ttgacaattaatcatcgg ctcgtataatgtgtgg**TT CGTGGACTAAGAAACGGT** gttttagagctagaaata gcaagt
SEQ ID NO: 24	NCg10157	mqo	ttgacaattaatcatcgg ctcgtataatgtgtgg**TG CAGGATTGTAGACAGGAG** gttttagagctagaaata gcaagt
SEQ ID NO: 25	NCg10248	asd	ttgacaattaatcatcgg ctcgtataatgtgtgg**TT CACCTCAGAGACGATTAG** gttttagagctagaaata gcaagt
SEQ ID NO: 26	NCg10437		ttgacaattaatcatcgg ctcgtataatgtgtgg**TG TTTGCTAAAGAGTAGGAA** gttttagagctagaaata gcaagt
SEQ ID NO: 27	NCg10463	SSADH	ttgacaattaatcatcgg ctcgtataatgtgtgg**AA CTCCCCGCGAAAGATCCG** gttttagagctagaaata gcaagt
SEQ ID NO: 28	NCg10521		ttgacaattaatcatcgg ctcgtataatgtgtgg**TT CGGAGACACACACATGTA** gttttagagctagaaata gcaagt
SEQ ID NO: 29	NCg10523	betB	ttgacaattaatcatcgg ctcgtataatgtgtgg**CC AGTGACTTTAGAGCTAGG** gttttagagctagaaata gcaagt
SEQ ID NO: 30	NCg10900	gapB	ttgacaattaatcatcgg ctcgtataatgtgtgg**CC AACTGATATCGTGCTGTA** gttttagagctagaaata gcaagt
SEQ ID NO: 31	NCg11526	gapA	ttgacaattaatcatcgg ctcgtataatgtgtgg**GT CGCCAGTGTATGCGTGAA** gttttagagctagaaata gcaagt
SEQ ID NO: 32	NCg12272	proA	ttgacaattaatcatcgg ctcgtataatgtgtgg**GC GCAGCAAAGCTACGTTTC** gttttagagctagaaata gcaagt

TABLE 4-continued

Primers for producing pCG9ts-series vectors and 13 randomly selected enzymes

SEQ ID NO	Gene	Annotation	Nucleotide sequence
SEQ ID NO: 33	NCg12578	vdh	ttgacaattaatcatcgg ctcgtataatgtgtgg**AT CGTCGTAAGGATTGATAT** gttttagagctagaaata gcaagt
SEQ ID NO: 34	NCg12619	gabD2/ ssadh	ttgacaattaatcatcgg ctcgtataatgtgtgg**GA GGTTATAGCGCCATTTAC** gttttagagctagaaata gcaagt
SEQ ID NO: 35	NCg12698	aid	ttgacaattaatcatcgg ctcgtataatgtgtgg**CT TGCCAATCCGATTAGAGC** gttttagagctagaaata gcaagt

2-2: Production of ssODN Each Binding to 13 Types of Genes

SsODN for deleting 13 arbitrary kinds of target genes was designed so that the site where the guide sequence of sgRNA binds was located between the two binding sequences of ssODN, and the total length was 80 nucleotides (Table 5). At this time, ssODN consists of a 5'-homology arm and a 3'-homology arm, and each homology arm is 40 base pairs, and was designed to bind to the outer parts of both ends of the target gene region including a sequence complementary to the guide sequence of sgRNA. When ssODN binds to the target, a loop structure is formed, and this part becomes a region where deletion occurs. The length of the deletion region was designed to have 100 base pairs so that deletion of the target gene could be easily detected through PCR.

TABLE 5

SsODN sequences binding to arbitrary 13 kinds of aldehyde dehydrogenase genes

SEQ ID NO	Nucleotide sequence
SEQ ID NO: 36	ggtgccatgggtgccaaaatgcgcaacatcggc gaagcttcgacgaaggcgtcaccgtgggccccc tggttgaggaaaaa
SEQ ID NO: 37	actggattgacggcgcgatttccccatccactt ccggcaagctgctaagacgtggggcaacctgtc tatcgctaagcgcc
SEQ ID NO: 38	gactgttgtggataactcttctgcttggcgcaa ggacgaccagtgctgaagccacttcacgatgcc gctggtcttgtaaa
SEQ ID NO: 39	gtcggtagcatcaaaagctcgcacgccgatgag tggccactcgccatcaatcagtgaacacccatg cagtgcggttg
SEQ ID NO: 40	ccacgattccacccagtggatgtccgcgctctc tgatgcacagagatcatccacctggaagctgga aaatccgttgcaga
SEQ ID NO: 41	gtaaccaccttgcttcgggtatagaagttgaaa gactcaggacttcgatgtccatctgaaattctc gagctgtacggcca
SEQ ID NO: 42	gtcgagagtactgacatgtctgcatcaggaagg ataatcgcttgtctactccgggtggcggacaa gggcatcaccgaaa

TABLE 5-continued

SsODN sequences binding to arbitrary 13 kinds of aldehyde dehydrogenase genes

SEQ ID NO	Nucleotide sequence
SEQ ID NO: 43	cttcgaagaatccgaaagcaccgacctgcgtgc cttcctgtcctggtttcccgcgaggcactgtat gacggtgctcgtct
SEQ ID NO: 44	aacgatgttgactgctgctgcacgtgcacgacg caggtcgttggtgcgaggcagttggtggtgcaa gatgcgccggagat
SEQ ID NO: 45	ctgcaggataccacgagcaggtgaggaatgcac agctcgcccaaaggcacacggacctgcacatct gaatgccgtt
SEQ ID NO: 46	ggcatcaacatcagcaatggaagcagtagcatc ggcgcaaatgagcagtcacaaggtctcctaaag agattgtgg
SEQ ID NO: 47	cccagaaagtgcaaaagcatgctcgacgtcagc tcatcatgaagacataggcagcggacctaaagg aagacgtttg
SEQ ID NO: 48	ggaatgatcttgtcggatgcagcgcggttgatc agcttgcgccctctgggatgagatcgccgatga tgttaatcagatac

Example 3: Production and Confirmation of *Corynebacterium glutamicum* with Inhibited 3-HP Production Ability and Improved 1,3-PDO Production Ability

3-1: Production of *Corynebacterium glutamicum* with Inhibited 3-HP Production Ability

The pCG9ts-ALD vectors and ssODN produced in Examples 2-1 and 2-2 were each transformed into wild-type *Corynebacterium glutamicum* (ATCC 13032) in order to delete the genes encoding arbitrary 13 kinds of aldehyde dehydrogenase that were expected to be involved in 3-HP biosynthesis from the genome. Then, for the transformed mutant *Corynebacterium glutamicum* strains, a pTacCC1-HrT vector (Korean Patent Application No. 2017-0042124; Cho et al., *Metabolic Engineering*, 42: 157-167, 201, SEQ ID NO: 57) and pCG9ts-ALD vectors were removed by curing on a 37° C. BHI plate. The strains produced through this process are shown in Table 6. However, the WAH3 strain and the WAH9 strain were not produced and the corresponding two genes are considered to be genes essential for cell survival.

TABLE 6

11 kinds of arbitrary aldehyde dehydrogenase-deleted *Corynebacterium glutamicum* strains

Name of strain	Genotype
WT	*C. glutamicum* ATCC 13032
WAH1	WT NCgl0049
WAH2	WT NCgl0157
WAH4	WT NCgl0437
WAH5	WT NCgl0463
WAH6	WT NCgl0521
WAH7	WT NCgl0523
WAH8	WT NCgl0900
WAH10	WT NCgl2272
WAH11	WT NCgl2578
WAH12	WT NCgl2619
WAH13	WT NCgl2698

3-2: Production of pEK-pduyE Vector for Construction of 1,3-PDO Biosynthetic Metabolic Pathway

In order to construct the 1,3-PDO biosynthetic metabolic pathway, *Klebsiella pneumoniae* DSMZ2026 (KCTC 4952) and *E. coli* W3110-derived foreign enzymes were expressed using the pEKEx1 shuttle vector of *Corynebacterium glutamicum* (Eikmanns et al., Gene 102: 93, 1991, SEQ ID NO: 58).

First, PCR was performed using the chromosomal DNA of the DSMZ2026 strain of *Klebsiella pneumoniae* as a template and primers of SEQ ID NOS: 49 and 50 to obtain a pduCDEGH gene cluster fragment (SEQ ID NO: 59) encoding glycerol dehydratase and glycerol reactivase. In order to ligate the obtained pduCDEGH gene fragment with the pEKEx1 vector as a shuttle vector, a pEK-pdu vector was produced by treatment with the restriction enzymes EcoRI and PstI, and then ligation using Gibson assembly (FIG. **4**).

Then, PCR was performed using the pTac15kyqhD recombinant vector (a recombinant vector (SEQ ID NO: 60) obtained by inserting yqhD derived from *E. coli* W3110 into a pTac15k vector (originated from p15A, tac promoter, KmR), as a template and primers of SEQ ID NOS: 51 and 52 to obtain a yqhD gene fragment encoding 1,3-PDO oxidoreductase.

In order to ligate the obtained gene fragment with the pEK-pdu vector, a pEK-pduyE vector was produced by treatment with the DraI restriction enzyme and ligation using Gibson assembly (FIG. **5**).

TABLE 7

Primers for producing pEK-pduyE vectors

SEQ ID NO	Nucleotide sequence
SEQ ID NO: 49	5'-ACAATTTCACACAGGAAACAGAATT CATGAGATCGAAAAGATTTGAAG-3'
SEQ ID NO: 50	5'-AAAACAGCCAAGCTTGGCTGCAGT TAAGCATGGCGATCCCGAAATG-3"
SEQ ID NO: 51	5'-TTCCAATGATGAGCACTTTT TTGACAATTAAT-3'
SEQ ID NO: 52	5'-GCGCCACATAGCAGAACTTTTTAG CGGGCGGCTTCGTATATAC-3'

3-3: Confirmation of Inhibition of 3-HP Production Ability and Improvement of 1,3-PDO Production Ability Through In Vivo Culture

Each strain prepared in Example 3-1 was transformed with the pCSglpFKD vector for constructing a glycerol-degrading metabolic pathway and pEK-pduyE for constructing a 1,3-PDO biosynthetic metabolic pathway. Then, selection was conducted on a BHIS plate medium (containing 37 g/L of brain heart infusion (BHI), 91 g/L of sorbitol and 15 g/L of agar) supplemented with 25 μg/L of Kanamycin and 200 μg/L of Spectinomycin. The 11 transformed mutant microorganisms were inoculated into a test tube containing 10 mL BHIS medium (containing 37 g/L of brain heart infusion (BHI) and 91 g/L of sorbitol) and pre-cultured at 30° C. for 16 hours. Then, 1 mL of the pre-cultured solution was inoculated into 25 mL of CGXII medium (Table 8) in a 250 mL baffle flask and cultured. The initial glycerol concentration was set to 40 g/L, and g/L of yeast extract in the medium was added, and flask culture was performed in triplicate for 48 hours.

TABLE 8

Components of CGXII medium used for culture of *Corynebacterium glutamicum*

Components of CGXII-glycerol medium	Concentration
$CaCl_2 \cdot 2H_2O$	13 mg/L
$FeSO_4 \cdot 7H_2O$	10 mg/L
$MnSO_4 \cdot 5H_2O$	14 mg/L
$ZnSO_4 \cdot 7H_2O$	1 mg/L
$CuSO_4 \cdot 5H_2O$	300 μg/L
$NiCl_2 \cdot 6H_2O$	20 μg/L
$(NH_4)_2SO_4$	20 g/L
Urea	2 g/L
KH_2PO_4	1 g/L
K_2HPO_4	1 g/L
Biotin	200 μg/L
Thiamine	500 μg/L
Protocatechuic acid	30 mg/L
MOPS	42 g/L
Glycerol	40 g/L
Spectinomycin	200 μg/L

The HPLC conditions used to measure the 3-HP concentration are as follows. First, an Agilent 1100 series HPLC instrument was used, and a DAD detector, an agilent MetaCarb 87H column, and another UV 210 nm detector were used as detectors and a column. At this time, 0.1% H3PO4 was fed as a buffer at a flow rate of 0.5 mL/min at 40° C. Next, Waters 1515 high performance liquid chromatography (Waters 1 Co., Milford, Mass., USA) was used for the measurement of 1,3-PDO. The detectors and column used herein were Waters 2414 refractive index detectors and A MetaCarb 87H column (300 by 7.8 mm; Agilent). At this time, 0.01N H2504 was fed as a buffer at a flow rate of 0.5 mL/min at 35° C.

As a result, as can be seen from FIGS. **6** and **7**, the strain transformed with the pCSglpFKD vector and the pEK-pduyE vector from the WAH13 strain the most inhibited 3-HP production and thus the most increased 1,3-PDO production. In addition, it can be seen that WAH1, WAH2, WAH5, WAH6 and WAH7 also exhibited inhibited 3-HP production and increased 1,3-PDO production.

INDUSTRIAL APPLICABILITY

The mutant *Corynebacterium glutamicum* according to the present invention can produce 1,3-PDO with high efficiency using inexpensive glycerol as a carbon source through inhibition of the ability to produce 3-HP, a by-product.

Although specific configurations of the present invention have been described in detail, those skilled in the art will appreciate that this description is provided to set forth preferred embodiments for illustrative purposes and should not be construed as limiting the scope of the present invention. Therefore, the substantial scope of the present invention is defined by the accompanying claims and equivalents thereto.

[Sequence Text]

An electronic file was attached.

SEQUENCE LISTING

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<213> ORGANISM: Artificial Sequence
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<210> SEQ ID NO 2
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 2

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<210> SEQ ID NO 3
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 3

aaggagatat agatggaaac caaagatctg at 32

<210> SEQ ID NO 4
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 4

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<210> SEQ ID NO 5
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 5

tctagaggcc cagccggcca ttataattag 30

<210> SEQ ID NO 6
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 6

ggatcccatg ctactcctac caaccaaggt 30

<210> SEQ ID NO 7
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 7

ttcgtggact aagaaacggt 20

<210> SEQ ID NO 8
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 8

tgcaggattg tagacaggag 20

<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 9

ttcacctcag agacgattag 20

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 10

tgtttgctaa agagtaggaa 20

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<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 11

aactccccgc gaaagatccg 20

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 12

ttcggagaca cacacatgta 20

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 13

ccagtgactt tagagctagg 20

<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 14

ccaactgata tcgtgctgta 20

<210> SEQ ID NO 15
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 15

gtcgccagtg tatgcgtgaa 20

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 16

gcgcagcaaa gctacgtttc 20

<210> SEQ ID NO 17
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

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<400> SEQUENCE: 17

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<210> SEQ ID NO 18
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 18

gaggttatag cgccatttac 20

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sgRNA

<400> SEQUENCE: 19

cttgccaatc cgattagagc 20

<210> SEQ ID NO 20
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for sgRNA-T1/TE fragment

<400> SEQUENCE: 20

tatagatatc ccgcggtata ttaattaata taaacgcaga aaggccc 47

<210> SEQ ID NO 21
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for sgRNA-T1/TE fragment

<400> SEQUENCE: 21

tggatgatgg ggcgattcag gtatagatat cttgacaatt aatcatcggc t 51

<210> SEQ ID NO 22
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for sgRNA-T1/TE fragment

<400> SEQUENCE: 22

aaggtgttgc tgactcatac caggtataga tatcccgcgg tata 44

<210> SEQ ID NO 23
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 23

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gagctagaaa tagcaagt 78

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<210> SEQ ID NO 24
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 24

ttgacaatta atcatcggct cgtataatgt gtggtgcagg attgtagaca ggaggtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 25
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 25

ttgacaatta atcatcggct cgtataatgt gtggttcacc tcagagacga ttaggtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 26
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 26

ttgacaatta atcatcggct cgtataatgt gtggtgtttg ctaaagagta ggaagtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 27
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 27

ttgacaatta atcatcggct cgtataatgt gtggaactcc ccgcgaaaga tccggtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 28
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 28

ttgacaatta atcatcggct cgtataatgt gtggttcgga gacacacaca tgtagtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 29
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 29

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ttgacaatta atcatcggct cgtataatgt gtggccagtg actttagagc tagggtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 30
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 30

ttgacaatta atcatcggct cgtataatgt gtggccaact gatatcgtgc tgtagtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 31
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 31

ttgacaatta atcatcggct cgtataatgt gtgggtcgcc agtgtatgcg tgaagtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 32
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 32

ttgacaatta atcatcggct cgtataatgt gtgggcgcag caaagctacg tttcgtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 33
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 33

ttgacaatta atcatcggct cgtataatgt gtggatcgtc gtaaggattg atatgtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 34
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 34

ttgacaatta atcatcggct cgtataatgt gtgggaggtt atagcgccat ttacgtttta 60

gagctagaaa tagcaagt 78

<210> SEQ ID NO 35
<211> LENGTH: 78
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pCG9ts-series vector preparation

<400> SEQUENCE: 35

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gagctagaaa tagcaagt 78

<210> SEQ ID NO 36
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 36

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gccccctggt tgaggaaaaa 80

<210> SEQ ID NO 37
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 37

actggattga cggcgcgatt tccccatcca cttccggcaa gctgctaaga cgtggggcaa 60

cctgtctatc gctaagcgcc 80

<210> SEQ ID NO 38
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 38

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gatgccgctg gtcttgtaaa 80

<210> SEQ ID NO 39
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 39

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acacccatgc agtgcggttg 80

<210> SEQ ID NO 40
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 40

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gctggaaaat ccgttgcaga 80

<210> SEQ ID NO 41
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 41

gtaaccacct tgcttcgggt atagaagttg aaagactcag gacttcgatg tccatctgaa 60

attctcgagc tgtacggcca 80

<210> SEQ ID NO 42
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 42

gtcgagagta ctgacatgtc tgcatcagga aggataatcg cttgtctact ccggggtggc 60

ggacaagggc atcaccgaaa 80

<210> SEQ ID NO 43
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 43

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ctgtatgacg gtgctcgtct 80

<210> SEQ ID NO 44
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 44

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gtgcaagatg cgccggagat 80

<210> SEQ ID NO 45
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 45

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tgcttcatct gaatgccgtt 80

<210> SEQ ID NO 46
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

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<400> SEQUENCE: 46

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tctcctaaag ttgattgtgg 80

<210> SEQ ID NO 47
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 47

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gacctaaagg aagacgtttg 80

<210> SEQ ID NO 48
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ssODN sequence

<400> SEQUENCE: 48

ggaatgatct tgtcggatgc agcgcggttg atcagcttgc gccctctggg atgagatcgc 60

cgatgatgtt aatcagatac 80

<210> SEQ ID NO 49
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pEK-pduyE preparation

<400> SEQUENCE: 49

acaatttcac acaggaaaca gaattcatga gatcgaaaag atttgaag 48

<210> SEQ ID NO 50
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pEK-pduyE preparation

<400> SEQUENCE: 50

aaaacagcca agcttggctg cagttaagca tggcgatccc gaaatg 46

<210> SEQ ID NO 51
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pEK-pduyE preparation

<400> SEQUENCE: 51

ttccaatgat gagcactttt ttgacaatta at 32

<210> SEQ ID NO 52
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer for pEK-pduyE preparation

<400> SEQUENCE: 52

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gcgccacata gcagaacttt ttagcgggcg gcttcgtata tac 43

<210> SEQ ID NO 53
<211> LENGTH: 3895
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: glpFKD

<400> SEQUENCE: 53

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ttgattttct tcggtgtggg ttgcgttgca gcactaaaag tcgctggtgc gtcttttggt 120

cagtgggaaa tcagtgtcat ttggggactg ggggtggcaa tggccatcta cctgaccgca 180

ggggtttccg gcgcgcatct taatcccgct gttaccattg cattgtggct gtttgcctgt 240

ttcgacaagc gcaaagttat tccttttatc gtttcacaag ttgccggcgc tttctgtgct 300

gcggctttag tttacgggct ttactacaat ttatttttcg acttcgagca gactcatcac 360

attgttcgcg gcagcgttga aagtgttgat ctggctggca ctttctctac ttaccctaat 420

cctcatatca attttgtgca ggctttcgca gttgagatgg tgattaccgc tattctgatg 480

gggctgatcc tggcgttaac ggacgatggc aacggtgtac cacgcggccc tttggctccc 540

ttgctgattg gtctactgat tgcggtcatt ggcgcatcta tgggcccatt gacaggtttt 600

gccatgaacc cagcgcgtga cttcggtccg aaagtctttg cctggctggc gggctggggc 660

aatgtcgcct ttaccggcgg cagagacatt ccttacttcc tggtgccgct tttcggccct 720

atcgttggcg cgattgtagg tgcatttgcc taccgcaaac tgattggtcg ccatttgcct 780

tgcgatatct gtgttgtgga agaaaaggaa accacaactc cttcagaaca aaaagcttcg 840

ctgtaatatg actacgggac aattaaacat gactgaaaaa aaatatatcg ttgcgctcga 900

ccagggcacc accagctccc gcgcggtcgt aatggatcac gatgccaata tcattagcgt 960

gtcgcagcgc gaatttgagc aaatctaccc aaaaccaggt tgggtagaac acgacccaat 1020

ggaaatctgg gccacccaaa gctccacgct ggtagaagtg ctggcgaaag ccgatatcag 1080

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tatgctgatg aacactggcg agaaagcggt gaaatcagaa aacggcctgc tgaccaccat 1740

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cgcatccatt cagtggctgc gcgatgaaat gaagttgatt aacgacgcct acgattccga 1860

atatttcgcc accaaagtgc aaaacaccaa tggtgtgtat gtggttccgg catttaccgg 1920

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cgtgctggaa gcgatgcagg ccgactctgg tatccgtctg cacgccctgc gcgtggatgg 2100

tggcgcagta gcaaacaatt tcctgatgca gttccagtcc gatattctcg gcacccgcgt 2160

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ccgtccaggc atcgaaacca ctgagcgtaa ttaccgttac gcaggctgga aaaaagcggt 2340

taaacgcgcg atggcgtggg aagaacacga cgaataaaag gagatataga tggaaaccaa 2400

agatctgatt gtgatagggg gcggcatcaa tggtgctggt atcgcggcag acgccgctgg 2460

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gatgcgtttt cgcctgccac atcgtccgca tctgcgcccg gcgtggatga ttcgcattgg 2700

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cgaagtgctt actcggactc gcgccacctc tgctcgccgc gaaaacggcc tgtggattgt 2940

ggaagcggaa gatatcgata ccggcaaaaa atatagctgg caagcgcgcg gcttggttaa 3000

cgccaccggc ccgtgggtga aacagttctt cgacgacggg atgcatctgc cttcgcctta 3060

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gttttccatc atcggcacta ccgatgtcga gtacaaaggc gatccgaaag cggtgaagat 3240

tgaagagagt gaaatcaatt acctgctgaa tgtgtataac acgcacttta aaaagcagtt 3300

aagccgtgac gatatcgtct ggacctactc cggtgtgcgt ccgctgtgtg atgatgagtc 3360

cgactcgccg caggctatta cccgtgatta cacccttgat attcatgatg aaaatggcaa 3420

agcaccgctg ctgtcggtat tcggcggtaa gctgaccacc taccgaaaac tggcggaaca 3480

tgcgctggaa aaactaacgc cgtattatca gggtattggc ccggcatgga cgaaagagag 3540

tgtgctaccg ggtggcgcca ttgaaggcga ccgcgacgat tatgccgctc gcctgcgccg 3600

ccgctatccg ttcctgactg aatcgctggc gcgtcattac gctcgcactt acggcagcaa 3660

cagcgagctg ctgctcggca atgcgggaac ggtaagcgat ctcggggaag atttcggtca 3720

tgagttctac gaagcggagc tgaaatacct ggtggatcac gaatgggtcc gccgcgccga 3780

cgacgccctg tggcgtcgca caaaacaagg catgtggcta aatgcggatc aacaatctcg 3840

tgtgagtcag tggctggtgg agtatacgca gcagaggtta tcgctggcgt cgtaa 3895

<210> SEQ ID NO 54
<211> LENGTH: 6538
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pCES208s-H36-S3

<400> SEQUENCE: 54

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caagagagag acccctacgg ggggaaccgt tttctgccta cgagatggca catttactgg 180

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gaagctttac ggcgtcctcg tggaagttca atgcccgcag acttaagtgc tctattcacg 240

gtctgacgtg acacgctaaa ttcagacata gcttcattga ttgtcgccac gagccagtct 300

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<223> OTHER INFORMATION: pUC19-sgRNA

<400> SEQUENCE: 55

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<220> FEATURE:
<223> OTHER INFORMATION: NCgl0049

<400> SEQUENCE: 56

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl0157

<400> SEQUENCE: 57

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl0248

<400> SEQUENCE: 58

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gcttcaggcg tcaagcttgt cgacgtccca accccacttg cagctgccgg cattgacgaa 900

tccctcgttg gacgcatccg tcaggactcc actgtcgacg ataaccgcgg tctggttctc 960

gtcgtatctg gcgacaacct ccgcaagggt gctgcgctaa acaccatcca gatcgctgag 1020

ctgctggtta agtaa 1035

<210> SEQ ID NO 59
<211> LENGTH: 1563
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl0437

<400> SEQUENCE: 59

atgatcaccg caaccgcact gcatgggtgt tcactgattg atggcgagtg ggtcgctgga 60

aaaaatggtg agattacagg attcgatccg cgcaccaatg cgagtctgaa cccttcctac 120

tctttagcaa acagcgcaca gctgcgcgcc gccacaacat cggcgaagcg agcttttgaa 180

agctaccgac tcactactcc agaggttaga gcagatttcc tggattccat cgctgacaac 240

atcgatgcgc tatccggcga gatcgtgcaa cgggcgagcc tggagacagg tttgggaact 300

acccgactca caggcgaagt agcccgcacc agcaaccagc tccgcctgtt tgcagaaacc 360

gtgagaagcg gacagttcca ccgagtacgc attgaacgag gaccgcggat tgatcttcgc 420

cagcgtcagg ttccgttggg accagtcgcg gtattcgggg caagcaactt ccccgtcgct 480

ttctctactg ctggtggcga tacagcatca gcgttggctg caggctgccc tgtggttttt 540

aaggcgcata atgcgcaccc tggaacagct gagctcgtcg ggcaagcggt gcggggagcc 600

gtcgaaaagc atgagtttga tgctggtgtg tttaaccttg tctacggccg tggcgtggaa 660

attggccagg agctggctgc ggatccgaat atcacggcaa tcggttttac cggttcacgc 720

cagggtggtt tggcactgtc acagactgcg tttagccgcc cagttcccgt tccagtcttt 780

gcagaaatga gtgccaccaa ccctgtgttc gtcttccccg gcgcgctggc ggatttggat 840

gcatcgagtt ccttggctga ggcgtttacc gcttccgtca ccggcagttc cgggcaattg 900

tgcaccaagc ctggcctcgt tttcatcccg cgcggtgttg ttggtgatgc ttttgtggcg 960

ctcgtagcag ccaaatttaa agaaaccacg ggtcaaacga tgctcacgca aggcatcgct 1020

caggcatggc agcgcggagt cgacaacctt gcagcacagc caagtgtaaa aatcctcgcc 1080

caaggcaccc ccggagatgg agagaacgcg ccgggcccgg tggtgtttga aagtgatgtg 1140

caggcgttgc taaataatgt ggtgttgcag gaagaaatct tcggtgcggc atcgctggtg 1200

gtgcgttatg attccccgga tcaactccac caagtagcca attcactcga gggacaatta 1260

acagccacga tccacgcatc ccaggatgat ttccaggaag tctcgaaact tatccccctc 1320

ttggaggatc tcgcgggccg tgttctttac ggcggctggc caacgggtgt ggaagttggg 1380

cacacggtta tccatggagg cccttatccg gcgacctcaa atgcgcagtc gacaagtgtt 1440

ggaaccctgg caatcgagag atttatgcgc ccggtttctt atcaaacttt cccggctgag 1500

ctgcttccag atccagtttc tgaggcgaat aaatgggctg tacctcggga aatagaccgt 1560

taa 1563

<210> SEQ ID NO 60

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<211> LENGTH: 1362
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl0463

<400> SEQUENCE: 60

gtgtctttga ccttcccagt aatcaacccc agcgatggct ccaccatcac cgagctagaa 60

aaccacgatt ccacccagtg gatgtccgcg ctctctgatg cagttgcagc tggtccttca 120

tgggctgcga aaactccccg cgaaagatcc gtggtactca ccgcaatctt cgaagcactg 180

accgaacgcg cccaagaact tgcagagatc atccacctgg aagctggaaa atccgttgca 240

gaagctcttg gtgaagtcgc ttatggtgca gaatacttcc gttggtttgc ggaagaagca 300

gtgcgcctgc ccggccgcta cggacagtca ccttccggaa tcggtcacat cgccgtcacc 360

cgcgcacccg tgggaccagt gctggcgatc accccatgga atttccccat cgccatggcc 420

acccgcaaaa tcgccccagc cctggccgct ggttgccccg tgttggtgaa acctgcttcc 480

gaaaccccac tgaccatggt caaagtgggg gagatcatcg cctccgtctt tgataccttt 540

aatatcccgc agggcttggt ctcaatcatc accaccactc gagatgcaga gctatcggca 600

gaactcatgg ctgatcctcg cttggctaaa gtcaccttca ctggatcaac caacgtggga 660

cgcatcctgg tccgccaatc cgcggaccga ctgctgcgca cctccatgga actcggcgga 720

aatgcagctt ttgttatcga cgaagccgca gacctcgacg aagccgtatc cggtgccatc 780

gccgcaaaac tccgcaacgc cggccaagta tgcatcgcag ctaaccgttt cttggttcat 840

gaatcccgcg ctgccgaatt cacctcaaag ctggcgacag ccatgcagaa cactcccatt 900

gggccggtga tttctgcccg ccaacgcgac cggatcgcag cactagtgga tgaagccatc 960

accgacggcg cccgcctcat catcggtggg gaggtccccg acggctccgg cttcttctat 1020

ccagccacca tcttggccga tgtccctgca cagtcacgga ttgtgcatga ggaaatcttc 1080

ggacctgtgg ccaccattgc cactttcacc gacttggccg aaggcgttgc acaagcaaat 1140

tccaccgaat tcggcctcgc agcctacgga ttcagcaaca atgtgaaagc aacacagtac 1200

atggcggaac acttggaagc cggaatggtc ggaatcaaca gaggcgccat ctctgaccca 1260

gcagcacctt ttggcggcat cggacaatcc ggcttcggca gagaaggcgg aaccgaagga 1320

atcgaagaat atctctccgt gcgttacctc gctttgccgt ga 1362

<210> SEQ ID NO 61
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl0521

<400> SEQUENCE: 61

ttggaaagtt ccaattctca ggcacaggga cgtgcggaca gaattctcga ttcagccttg 60

aaagaaggcg cttcaatagt tgttgatggc cgtacagctc gagaatttca gatggacatc 120

gaagtcggaa tggttggcat taacgtgcca atcccagtcc caattggcgc tttctcattt 180

ggaggttgga aagactcact attcggagac acacacatgt atggatctga gtctttcaac 240

ttctataccc gaagcaaggt ggttaccact cgctggcctc ttccaaatga atcacagatt 300

gagcttggct tccccaccca ctaa 324

<210> SEQ ID NO 62
<211> LENGTH: 1494

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl0523

<400> SEQUENCE: 62

atgaatgctg caaccaggcg tgcttctctg caactcccct atacccatgt cgatgatttc 60

tacatcaacg gttcctgggt taaagcagaa ggaacacaac gcaaccccgt agttgatcct 120

gcggtcggtc aagaatgggg atctgttcca gaagcaaccg catctgaatt ggactctgcg 180

gtgggagctg cacgtacagc gctaaagtcg tggagtgcac ttacaggtgc ggaacgaaca 240

ggctacctcc tgaaaatcgc gacggaaatt gaatcccgtt ctgaagctct agcacttact 300

aatacccgcg aaaatggttc ccccatttcc gagacccgtg gagctgcgtc caatgcagca 360

ggaattttcc gttactttgc cactctcgcg ccttggttag acggcgaaga catccgccca 420

tttcctgccg gtagcgccga atccatcgtg gataaagatc ccatcggtgt ctgcgcactc 480

atcgccccat ggaatttccc gatcaacctt gtagtcatca aactggcacc agcacttctt 540

gccggctgta ccgtcatcat caaaccagcc tcccccaccc cactgtcgat ccgtttcatc 600

atcgaagcca tcgaagccgc cggagtgcca gcaggcgtag tcaacctact caccggttca 660

gggcgtttcg gtgatgccct tgtccgccac cccggagtag acaaggtagc gtttaccgga 720

tcaacgcctg ttggaaagaa gatcgctgcc gcctgcggag aactactccg accagtgact 780

ttagagctag gcggaaaatc ttccgcgatt atccttcctg atgcagacat gtcagtactc 840

tcgacgcggt tgattcgatc ctgtatgcgc aacactggac aaacctgcta catcagtacc 900

cggattattg cccctagctc acgctatgcg gaagtcgtac aaacagtggc aagcactatc 960

gctgcaggta gacaaggtga cccctatgat gaagaaacgg tttttgggcc agttgccagc 1020

gcctctcagt actcaaccgt catgtcttac attgactccg cacgagagga aggtgcacga 1080

gtggttgcag gtggaacccg gtcaatcagc ctttctgaag gtttagaatc aggcgagttt 1140

atccaaccaa ccgtgtttgc cgatgtcacc cccgacatgc ggatatcacg cgaagaaatc 1200

ttcggccctg ttatttccat cctaaagtac gacgatacaa acggtgtttc cgaagcaatc 1260

gcactagcca acaacacgaa attcggtctc ggtggcttgg tatttggtgc ggatgaggaa 1320

caagcactag aagtcgcccg tcaagtggat tctggttccg taggcatcaa cttcttcggt 1380

tccaaccatt ccgccccatt tggaggacgc cacgaatccg gtatgggagt ggaatacggc 1440

atcgaaggcc tcagtgctta cctgacatac aagagtattc accgaaccat ttag 1494

<210> SEQ ID NO 63
<211> LENGTH: 1443
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl0900

<400> SEQUENCE: 63

atgacgcaca accacaagga ctggaacgat cgcattgcag ttgcggagga aatggtgccg 60

ttgatcgggc gcctgcaccg caacaacaac gtggtggttt ccgtattcgg tcgtctcctt 120

gtgaatgtct cagacatcga tatcatcaag tctcaccgct acgcccgcca catcatatcc 180

aaggaacttc cactggaaag ctccttggat attttgcgcg aactggtaga tatgaacctt 240

ggtaccgcat cgatcgacct gggacagctg gcctacagct tcgaagaatc cgaaagcacc 300

gacctgcgtg ccttcctgga ggacgctctc gcgccggtca ttggtgcgga aaccgacatc 360

-continued

aacccaactg atatcgtgct gtacggtttc ggccgcatcg gtcgcctgct ggcccgcatc 420

ctggtttccc gcgaggcact gtatgacggt gctcgtctgc gcgccatcgt ggtccgcaaa 480

aatggtgaag aagacctggt caagcgcgca tccttgctgc gtcgtgattc tgtccacggt 540

ggattcgatg gcaccatcac caccgattat gacaacaaca tcatctgggc caacggcacc 600

ccaatcaagg tcatctactc caatgaccca gccaccattg attacaccga atacggcatc 660

aatgacgccg tcgtggtaga caacaccggc cgctggcgtg accgcgaagg cctgtcccag 720

cacctcaagt ccaagggcgt tgccaaggtt gtactcaccg cgccgggcaa gggcgatctg 780

aagaacatcg tgtacggcat caaccacacc gacatcaccg cagatgatca gatcgtttcc 840

gcagcatcat gcaccaccaa tgccattacc ccagtgctca aggtgatcaa tgatcgctac 900

ggcgtggaat tcggccacgt agaaaccgtt cactccttca ccaatgacca gaacctgatc 960

gacaacttcc acaagggttc tcgccgtggt cgcgcagcag gtctgaatat ggttctcacc 1020

gaaaccggcg ctgcaaaggc tgtatccaag gcgcttccag agctggaagg caagctcacc 1080

ggcaatgcca tccgcgttcc tacccctgac gtgtccatgg ctgtgctcaa cttgaccctg 1140

aacacggagg tggaccgcga tgaggtcaac gagttcctcc gccgtgtgtc cctgcactct 1200

gacttgcgcc agcaaatcga ctggatccgt tccccagagg ttgtttccac tgacttcgtg 1260

ggcaccaccc acgcgggcat cgttgatggt ctagccacca tcgcaaccgg tcgccacctg 1320

gtgctttacg tgtggtacga caacgagttc ggctactcca accaggtcat tcgcatcgtc 1380

gaggagatcg ccggcgtgcg tcctcgcgtg tacccggagc gcaggcagcc agccgtacta 1440

tag 1443

<210> SEQ ID NO 64
<211> LENGTH: 1005
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl1526

<400> SEQUENCE: 64

atgaccattc gtgttggtat taacggattt ggccgtatcg gacgtaactt cttccgcgca 60

gttctggagc gcagcgacga tctcgaggta gttgcagtca acgacctcac cgacaacaag 120

acccttcca cccttctcaa gttcgactcc atcatgggcc gccttggcca ggaagttgaa 180

tacgacgatg actccatcac cgttggtggc aagcgcatcg ctgtttacgc agagcgcgat 240

ccaaagaacc tggactgggc tgcacacaac gttgacatcg tgatcgagtc caccggcttc 300

ttcaccgatg caaacgcggc taaggctcac atcgaagcag gtgccaagaa ggtcatcatc 360

tccgcaccag caagcaacga agacgcaacc ttcgtttacg gtgtgaacca cgagtcctac 420

gatcctgaga accacaacgt gatctccggc gcatcttgca ccaccaactg cctcgcacca 480

atggcaaagg tcctaaacga caagttcggc atcgagaacg gcctcatgac caccgttcac 540

gcatacactg gcgaccagcg cctgcacgat gcacctcacc gcgacctgcg tcgtgcacgt 600

gcagcagcag tcaacatcgt tcctacctcc accggtgcag ctaaggctgt tgctctggtt 660

ctcccagagc tcaagggcaa gcttgacggc tacgcacttc gcgttccagt tatcaccggt 720

tccgcaaccg acctgacctt caacaccaag tctgaggtca ccgttgagtc catcaacgct 780

gcaatcaagg aagctgcagt cggcgagttc ggcgagaccc tggcttactc cgaagagcca 840

ctggtttcca ccgacatcgt ccacgattcc cacggctcca tcttcgacgc tggcctgacc 900

aaggtctccg gcaacaccgt caaggttgtt tcctggtacg acaacgagtg gggctacacc 960

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tgccagctcc tgcgtctgac cgagctcgta gcttccaagc tctaa 1005

<210> SEQ ID NO 65
<211> LENGTH: 1299
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl2272

<400> SEQUENCE: 65

atgagttcaa cgaccctaac tgatgaccaa attcgcgaca atgagcggac cgaagttcta 60

gctaaagcaa ctgcagctaa gaacatcgtc ccggatattg cagtgttggg caccggaccg 120

aagaacgcaa tcctgcgtgc ggcggcagat gaactcgttg cacgcagcgc agaaatcatc 180

gaagccaacg cttccgatat cgaagcgggt cgcgcaaacg gcatggaaga atccatgatt 240

gatcgccttg cccttgatga atctcgcatt gagggcatcg ctggcggttt gcgccaggtt 300

gctggcctga ccgacccagt gggtgaagta ctgcgcggac atgtcatgga aaacggcatt 360

cagatgaagc aggtccgtgt gcctttgggc gtgatgggca tggtctatga agcccgccct 420

aacgtcaccg tcgacgcctt cggcctggca ctcaagtccg gaaacgtagc tttgctgcgc 480

ggttcctcca cagctgtgca ttccaacacc aagctcgtgg aaatcctgca ggacgtcctc 540

gagcgtttcg agctgccacg cgaaaccgtg cagttgctgc cttgccaaac ccgcggatcc 600

gtccaagatt tgatcaccgc acgcggcctc gttgacgtgg tcatcccacg cggcggcgca 660

ggactaatca acgcagtggt caccggtgcg accgtgccca ccattgaaac cggcaccggc 720

aactgccact tctacatcga tgccgaagcc aagcttgatc aggcaatcgc catggtcatc 780

aacggcaaga cccgccgctg cagcgtgtgc aacgctactg aaaccgcgct tctcgacgcc 840

gccctcagcg actcagacaa gcttgcagtc gtccaggcgc tccaggaagc aggagtcaca 900

attcatggac gggtggccga attggaagca ttcggtgcaa ccgacgtggt ggaagcaact 960

gaaactgact gggattctga gtacctgtcc ttcgatatcg ctgtcgctgt ggttgacggt 1020

gtggatggag ctctggcaca catcgctaag tacagcacca agcacaccga agcgatcgcc 1080

acccaaaaca ttgaaaccgc tcagcgcttt gcagatcgcg tcgatgcagc agcggtgatg 1140

ataaacgcat ccaccgccta caccgatggg gagcagtacg gcatgggcgc ggagatcggc 1200

atttccaccc agaaactgca tgcacgtgga ccaatggccc tgccagagct gacctccacc 1260

aagtggattc tgcagggcac aggacaaatt aggccttaa 1299

<210> SEQ ID NO 66
<211> LENGTH: 1455
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl2578

<400> SEQUENCE: 66

gtgactgcaa catttgctgg aatcgacgcc accaaacacc tcatcggagg tcagtgggtg 60

gagggaaact cggatcgaat ttccaccaat atcaatcctt acgacgattc cgtaatcgcc 120

gaaagcaaac aagcttccat tgctgatgtt gatgccgcgt atgaagccgc gaagaaggcc 180

caggctgagt gggcagctac gcccgctgcg gaacgatctg ccatcatcta ccgtgcggct 240

gaacttcttg aagagcaccg ggaggaaatc gtggaatggc tgatcaagga atccggctcg 300

acgcgttcca aggctaattt ggaaatcact ttggcaggaa acatcactaa agaatcggct 360

-continued

tcattccctg gtcgtgtgca tggtcgaatt tctccttcga atactccggg caaagaaaac 420

cgtgtgtacc gcgtagccaa gggcgttgtc ggagtgatta gtccatggaa tttcccactg 480

aacctctcga tccgctcggt tgctccggca ctagccgtgg gcaacgccgt agtgattaag 540

cctgcgagtg ataccccagt tactggtggt gtaattcctg cacgaatctt tgaggaggcc 600

ggagttcctg caggcgtgat cagcacggtt gcgggcgcag gatctgaaat cggtgatcac 660

tttgtcaccc acgccgtgcc aaagctgatt tctttcaccg gttcaacccc agtcggtcgt 720

cgtgtcggtg agctggcaat taatggtgga ccaatgaaaa ctgttgcact agagctcggt 780

ggcaacgcgc cgttcgttgt gcttgccgac gccgacatcg acgccgctgc ccaggctgcc 840

gcagttggcg ctttcctaca ccagggacag atttgtatgt caatcaaccg agtcattgtt 900

gatgctgcag ttcatgatga attcctagag aagttcgttg aagcagtgaa gaacattcca 960

accggcgatc caagcgcaga aggaaccctt gttggacctg tcattaatga cagtcagctc 1020

agtggtttga aggaaaagat cgagttggcc aaaaaggaag gcgcaaccgt ccaggttgaa 1080

gggccaattg aaggccgact ggttcatccg catgtgttct ctgatgtcac ctctgacatg 1140

gaaatcgctc gtgaggaaat cttcggacct ctcatcagcg tgctgaaggc cgatgatgag 1200

gcacacgcag cagagctggc caatgcttcc gactttggtt tgagcgcggc agtgtggtcg 1260

aaggatattg atcgtgcagc ccagtttgct ctgcagattg attccggcat ggttcacatc 1320

aatgacctca ccgtcaacga tgaaccacac gtgatgttcg gtggttcaaa gaactctggc 1380

ctcggccgct tcaacggcga ttgggcgatc gaggagttca ccacagatcg atggatcggc 1440

atcaagcgca gctaa 1455

<210> SEQ ID NO 67
<211> LENGTH: 1566
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl2619

<400> SEQUENCE: 67

atgatcaaac gtcttccttt aggtccgctg cctaaagaac ttcatcagac tctgcttgat 60

ctgaccgcaa atgcccaaga tgcggcgaaa gtggaggtta tagcgccatt tactggcgag 120

accctcggat ttgtttttga tggtgatgag caagacgtcg agcatgcttt tgcactttca 180

agggcagccc agaaaaagtg ggtgcacacc acggcagtgg aacggaagaa gatcttcctg 240

aagtttcatg atctggtatt gaaaaaccgt gagctgctca tggacatcgt gcagttggaa 300

acaggcaaaa atcgagcatc ggctgccgat gaggtgttgg acgttgcgat caccacccgc 360

ttctacgcaa acaatgcagg aaagttttta aatgacaaga aacgccccgg cgcgcttccg 420

atcatcacga aaaacacaca acagtatgtg cccaagggag tggtcgggca gatcacgccg 480

tggaattacc ctttaacttt gggagtatct gatgctgttc cggcgctgct ggcaggaaac 540

gcagtggtgg ctaaacctga cctcgcgaca cctttctcct gcttgatcat ggtgcacctg 600

ctcattgaag ccggtctgcc gcgtgatttg atgcaggttg tcaccggccc tggcgatatt 660

gttggcggtg cgattgcagc tcagtgtgat ttcctcatgt tcactggatc cacggccacg 720

ggccggatct tgggtcggac aatgggtgag cgtttggtgg gtttctctgc ggaattaggc 780

ggaaagaacc ctcttattgt ggccaaggat gcagatctgg acaaggtgga agctgagctt 840

ccgcaggcgt gtttttccaa ctcggggcaa ttgtgtgtct ccactgaacg tatttatgtc 900

gaggaagacg tgtacgagga ggtgattgca cggtttagca aggcggcgaa agccatgtcc 960

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gatcgggtga gcacctttgt tgatcaggct aaagctgcgg gcgccacggt gctgtgcggt 1080

ggcaagtcac gccctgatat tggtcccttc ttctatgagc ccacggtatt ggcggatgtc 1140

ccagagggca ccccactgct cacggaggaa gtcttcgggc cggtggtgtt catcgaaaag 1200

gtagccacac tggaagaagc cgtcgataag gcaaatggca cgccctacgg cctgaatgcg 1260

tccgtctttg ggtcgtcgga aaccggcaat cttgttgcag gccagctgga agctggcggt 1320

atcggtatta atgatggcta cgccgcgacg tgggcgagcg tgtccacgcc tctgggtggc 1380

atgaagcagt cggggctggg gcaccgccat ggtgcggagg gaattacaaa atatgcggag 1440

atccgaaaca tcgcggagca gcgctggatg tctatgcgtg ggccggccaa aatgccgcga 1500

aaggtgtact cagacaccgt ggccacagcg ctaaagctgg gcaaaatctt taaagttttg 1560

ccgtag 1566

<210> SEQ ID NO 68
<211> LENGTH: 1521
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: NCgl2698

<400> SEQUENCE: 68

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gagaactaca ttggtggcaa gtgggttcca ccggtagagg gccagtacct tgagaacatt 120

tcacctgtca ctggtgaagt tttctgtgag gtcgcacgtg gcaccgcagc ggacgtggag 180

cttgcactgg atgctgcaca tgcagccgct gatgcgtggg gcaagacttc tgtcgctgaa 240

cgtgctctga tcctgcaccg cattgcggac cgcatggaag agcacctgga agaaatcgca 300

gttgcagaaa cctgggagaa cggcaaggca gtccgtgaga ctcttgctgc agatatccca 360

ctggcaatcg accacttccg ctactttgct ggcgcgatcc gtgctcagga agatcgttcc 420

tcacagatcg accacaacac tgttgcttac cacttcaacg agccaatcgg tgttgttggt 480

cagatcattc cttggaactt cccaatcctc atggctacct ggaagctcgc accggcactt 540

gctgcaggta acgcgatcgt catgaagcca gctgagcaga ccccagcatc cattttgtat 600

ctgattaaca tcatcggcga tctcatccca gagggcgtcc tcaacatcgt caacggactc 660

ggcggtgaag caggcgctgc actgtccggc tctaatcgga ttggcaagat tgctttcacc 720

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atgggtgctc aggcgtccca ggagcagatg gacaagatct cctcctacct gaagatcggc 1080

ccagaagaag gcgctcaaac cctcactggt ggcaaggtca acaaggttga tggcatggag 1140

aacggttact acattgagcc aaccgttttc cgcggcacca acgacatgag gatcttccgc 1200

gaggaaatct tcggaccagt cctttctgtt gctaccttca gcgacttcga tgaggccatc 1260

cgtattgcaa acgacaccaa ctacggcctc ggcgctggtg tctggagccg tgaccaaaac 1320

accatttatc gtgcaggtcg cgcaatccag gctggtcgag tttgggtcaa ccagtaccac 1380

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aactacccag cgcactccgc tttcggtgga tacaaggagt ccggcatcgg ccgtgagaac	1440
cacctcatga tgctgaacca ctaccagcag accaagaacc tgttggtctc ctacgatcca	1500
aacccaaccg gactgttctg a	1521

The invention claimed is:

1. A mutant *Corynebacterium glutamicum* cell that has been genetically modified by (i) introducing the *E. coli* glpF gene, the *E. coli* glpK gene, the *E. coli* glpD gene, the *E. coli* yqhD gene, and the *K. pneumoniae* pduCDEG operon, and (ii) disrupting an endogenous gene encoding an aldehyde dehydrogenase that comprises comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 56, 57, 59, 60, 61, 62, 63, 65, 66, 67, and 68.

2. The mutant *Corynebacterium glutamicum* cell according to claim **1**, wherein said genes comprise a strong promoter selected from the group consisting of tac, trc and tuf.

3. A method of producing 1,3-propanediol (1,3-PDO) from glycerol comprising:

(a) culturing the mutant *Corynebacterium glutamicum* cell according to claim **1** in a glycerol-containing medium to produce 1,3-PDO; and

(b) collecting the produced 1,3-PDO.

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